PATENT APPL. 09/836,750
Docket No. 1000-10-CO1

CERTIFICATE OF MAILING

I hereby certify that the attached DECLARATION OF ANDREW E. LORINCZ, M.D. was delivered to the Assistant Commissioner for Patents by the undersigned from Arrow Intellectual Property Services, 2001, Jefferson Davis Highway, Suite 602, Arlington, Virginia 22202, by hand carrying said DECLARATION to Art Unit 1646, Crystal Plaza 1, Tenth Floor, Attention: Examiner Elizabeth C. Kemmerer this 17th day of June, 2003.

Dated: June 17, 2003

Ann Rutledge
Printed Name: Ann Rutledge

ARROW INTELLECTUAL PROPERTY SERVICE

)ocket No./ 1000-10-C01
·erial No.: 09/836,750
'iled/ 04/17/01
)ue Date:

'he Patent Office acknowledges, and has stamped hereon, the date of receipt f the items check below:

- ☐ Transmittal Letter
- ☐ Application – Trademark
- ☐ Application – Patent Specification Total Pgs
- ☐ Total Claims Ind. Claims Total Pgs
- ☐ Fee: $
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- ☐ Drawings: Formal Informal Total Pgs
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- ☐ Information Disclosure Statement
- ☐ Form PTO-1449 References Total No
- ☐ Request for Extension of Time Fee: $
- ☐ Amendment/Response
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Docket No. 1000-10-CO1
LORINCZ DECLARATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: James P. Elia	)	
	)	
SERIAL NO.: 09/836,750	)	EXAMINER: E.C. Kemmerer, Ph.D.
	)	
FILED: April 17, 2001	)	
	)	GROUP ART UNIT: 1646
FOR: METHOD AND APPARATUS	)	
FOR INSTALLATION OF	)	
DENTAL IMPLANT	)	

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DECLARATION OF ANDREW E. LORINCZ, M.D.

I, Andrew E. Lorincz, declare as follows:

1. I reside at 3628 Belle Meade Way, Mountain Brook, Alabama 35223.

2. My Curriculum Vitae is attached hereto as Exhibit A.

3. I have read and understood the disclosures of the above-referenced patent application at page 20, line 10 through page 21, line 15; and page 44, line 19 through page 46, line 16. A copy of such disclosures is attached hereto as Exhibit B.

4. I note that the disclosures referenced in above Paragraph 3 relate to using a growth factor for promoting the growth of soft tissue and, more specifically, to a method of using a growth factor for growing muscle in a human heart.

5. I am aware of and have considered the definition of *growth factor* in the specification of the above-referenced patent application at page 20, line 10 through page 21, line 15. Such definition is set forth in Exhibit C along with a definition from the medical dictionary, MEDLINE plus: Merriam-Webster Medical Dictionary. A service of the U.S. NATIONAL LIBRARY OF MEDICINE and the NATIONAL INSTITUTES OF HEALTH. I find that the dictionary definition is consistent with that contained at page 20, line 10 through page 21, line 15 of the above-referenced patent application. I believe that both definitions are appropriate for use in the field of tissue growth and would be understood by one skilled in the medical arts. Accordingly, I am adopting and utilizing the definition contained in the patent application throughout this declaration.

6. I have read and understood the claims set forth in Exhibit D and have been informed that such claims will be presented to the Patent and Trademark Office in the near future.

7. The materials included in attached Exhibit E illustrate that placement of a growth factor in a human patient causes muscle growth in a heart. These materials report work performed by reputable, skilled scientists and reputable organizations in the medical arts. Consequently, I believe that these reports would be recognized as clearly valid by one of ordinary skill in the medical arts because they report the results of scientific tests conducted by competent, disinterested third parties with use of proper scientific controls.

8. Based upon above Paragraphs 3-7, it is my opinion that introducing a growth factor into a human patient will predictably cause new muscle growth in the heart of the patient.

9. Based upon above Paragraphs 3-6, it is my opinion that one skilled in the medical arts, armed with the knowledge in such paragraphs, would be able to practice the method set forth in Exhibit D without need for resorting to undue experimentation.

10. Declarant states that the above opinion was reached independently.

Declarant understands that (1) any willful false statements and the like made herein are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon, and (2) that all statements made of Declarant's own knowledge are true and that all statements made on information and belief are believed to be true.

Further Declarant sayeth not.

Date: 6-9-03

Andrew E. Lorincz

Andrew E. Lorincz

EXHIBIT A

CURRICULUM VITAE

NAME:	Andrew E. Lorincz, M.D.
TITLE:	Professor of Pediatrics University of Alabama at Birmingham
SOCIAL SECURITY:	361-16-4853
ADDRESSES:	University of Alabama at Birmingham Mortimer Jordan Hall B-70 1825 University Boulevard Birmingham, Alabama 35294-2010 Telephone: (205) 934-7038 FAX: (205) 975-9147 Email: aelorincz@vprua.vprua.UAB.edu

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3628 Belle Meade Way
Mountain Brook, Alabama 35223
Telephone: (205) 967-4678

BIRTH: 5/17/26 Chicago, Illinois

MARITAL STATUS: Married, 12/14/65 - Diane DeNyse Lorincz

EDUCATION:

1948-1952	University of Chicago, School of Medicine, M.D. Degree
1948-1950	University of Chicago, B.S. Degree (Anatomy & Biochemistry)
1946-1948	University of Chicago, Ph.B. Degree

POSTDOCTORAL EDUCATION:

Jan-Mar 1980	Lysosomal Storage Disease Laboratory, Eunice Kennedy Shriver Center, Waltham, MA (Harvard), Visiting Scientist
1955-1956	LaRabida Jackson Park Sanitarium, University of Chicago, Junior Staff Physician Department of Pediatrics, University of Chicago Clinics Bob Roberts Memorial Hospital
1955-1958	Arthritis and Rheumatism Foundation Fellow
1954-1955	Benjamin J Rosenthal Clinical and Research Fellow
1953-1954	Junior Assistant Resident
1952-1953	Intern

ACADEMIC APPOINTMENTS:

1996-present	Professor Emeritus, Department of Pediatrics
1984-1996	School of Public Health, University of Alabama at Birmingham, Professor
1971-1996	University of Alabama at Birmingham, Member of Graduate Faculty
1968-1996	University of Alabama at Birmingham, Professor of Pediatrics
1971-1984	Division of Engineering Biophysics, University of Alabama at Birmingham, Associate Professor
1968-1982	University of Alabama at Birmingham, Associate Professor of Biochemistry
1976- 1980	School of Optometry, University of Alabama at Birmingham, Professor Optometry
1971-1980	School of Nursing, University of Alabama at Birmingham, Clinical Associate Professor
1970-1980	Center for Developmental and Learning Disorders, University of Alabama at Birmingham, (A University Affiliated Facility for Developmental Disability), Director
1970-1976	School of Optometry, University of Alabama at Birmingham, Associate Professor of Pediatric Optometry
1966-1968	Medical Teaching and Research, Unit of the University of Florida at the Sunland Training Center, Gainesville, Florida, Director
1963-1968	Department of Surgery (Orthopaedics), University of Florida College of Medicine, Gainesville, Florida, Research Associate Professor
1962-1968	Department of Pediatrics, University of Florida College of Medicine, Gainesville, Florida, Associate Professor
1959-1962	Department of Pediatrics, University of Florida College of Medicine, Gainesville, Florida, Assistant Professor
1956-1959	Department of Pediatrics, University of Chicago School of Medicine, Chicago, Illinois, Instructor

PROFESSIONAL LICENSES - PHYSICIAN AND SURGEON:

5/26/69	State of Alabama
8/10/59	State of Florida (inactive)
9/22/54	State of Illinois (inactive)

SPECIALTY CERTIFICATION:

May 1958	American Board of Pediatrics, Diplomate

BOARDS, COMMITTEES AND CONSULTANTSHIPS:

1994-present	Board member of The Mental Retardation and Developmental Disabilities, Health Care Authority of Jefferson County, Inc.
1991-present	Editorial Board for the Annals of Clinical and Laboratory Science, Member
1988-present	Medical Advisory Board of the National MPS Society, Member
1980-1986	Mental Retardation, Consulting Editor
1979-present	National Tay-Sachs and Allied Diseases Association, Scientific Advisory Committee, Member
1978-present	Mayor's Council of Disability Issues
1979-1984	Osteogenesis Imperfecta Foundation, Inc., Board Member Alabama O.I.Chapter
1974-1981	Child Mental Health Services, Inc., Birmingham, Alabama, Board Member
1977-1978	Elizabethtown Committee on Planning and Evaluation, Legislative Committee, State of Pennsylvania
1973-1975	Human Rights Committee for the Partlow State School and Hospital, Tuscaloosa, Alabama, Member - Federal Court Appointed
1971-1974	American Academy of Pediatrics, Committee on Children With Handicaps
1971-1973	American Journal of Mental Deficiency, Consulting Editor
1965-1973	Head Start, Medical Consultant
1967-1972	Journal of Investigative Dermatology, Editorial Consultant
1961-1968	Sunland Hospital, Orlando, Florida, Medical and Research Consultant
1965-1966	State of Florida Interagency Committee on Mental Retardation Planning, Co-Chairman, Mental Retardation Research Committee Alabama Developmental Disabilites Planning Council
1982-1984	Maternal and Child Health, Member of Advisory Committee
1979-1984	Member (Secretary, 1980; Vice Chairman, 1984)
1973-1979	Consultant

American Association of University Affiliated Facilities

1975-1978	American Association of University Affiliated Programs for the Developmentally Disabled, Board Member

American Association on Mental Retardation

1980,84,85	Prevention Committee, Chairman
1980-1982	Member of Council
1978-1980	Medicine Division and Member Executive Committee, Vice President

BOARDS, COMMITTEES AND CONSULTANTSHIPS: (CONT'D)

Association of Retarded Citizens of Jefferson County

1990-present	Board Member
1975-1985	Board Member
1977-1978	Second Vice President
1980	Recipient of Distinguished Service Award

PROFESSIONAL SOCIETIES:

American Academy for Cerebral Palsy and Developmental Medicine, (Fellow)
American Academy on Mental Retardation (President Elect, 1975-76; President, 1976-77)
Emeritus Member
American Academy of Pediatrics (Fellow)
American Association for the Advancement of Science
American Association for Clinical Chemistry, Inc.
American Association on Mental Retardation (Fellow)-Life Member
American Chemical Society
American Federation for Clinical Research
American Medical Association
American Society for Human Genetics
American Society for Investigative Pathology
Association of Clinical Scientists
International Society for Mycoplasmology
Jefferson County Pediatric Society
Society for Complex Carbohydrates
Society for Investigative Dermatology
Society for Pediatric Research
Society for Sigma Xi
Southern Society for Pediatric Research (President, 1964)

BIBLIOGRAPHY

1. Dorfman, A.; Lorincz, A.E.: Occurence of Urinary Acid Mucoploysaccharides in the Hurler Syndrome. Proceedings of the National Academy of Science, 43:443-46, 1957.
2. Dorfman, A., Gross, J.I.; Lorincz, A.E.: The Treatment of Acute Rheumatic Fever. Pediatrics, 27:692-705, May, 1961.
3. Lorincz, A.E.: Heritable Disorders of Acid Mucopolysaccharide Metabolism in Humans and in Snorter Dwarf Cattle. Annals of the New York Academy of Science, 91:644-58, 1961.
4. Shepard, T.H., Lorincz, A.E., Gartler, S.M.: Desulfuration of Thiourea by Saliva. Proceedings of the Society of Experimental Biology and Medicine, 112:38-42, July, 1963.

BIBLIOGRAPHY (CONT'D)

5. Lorincz, A.E.: A Heritable Disorder of Acid Mucopolysaccharide Metabolism in Cattle Which Resembles the Hurler Syndrome. Proceedings of the Second International Congress on Mental Retardation, Vienna, 1961, Part I: 70-74, 1963.

6. Gennaro, J.F., Jr., Callahan, W.P., III, Lorincz, A.E.: The Anatomy and Biochemistry of a Mucus-secreting Cell Type Present in the Poison Apparatus of the Pit Viper, Ancistrodon Piscivorus Piscivorus. Annals of the New York Academy of Sciences, 106:463-71, March, 1963.

7. Wolfson, S.L., Davidson, E., Harris, J.S., Kahana, L., Lorincz, A.E.: Long-term Corticosteroid Therapy in Hurler Syndrome. American Journal of Diseases in Children, 106:3-10, July, 1963.

8. Wainer, A., Lorincz, A.E.: Studies of Mercapturic Acid Synthesis by Humans. Life Sciences, No.7:504-08, 1963.

9. Lorincz, A.E.: Hurler's Syndrome in Man and Snorter Dwarfism in Cattle: Heritable Disorders of Connective Tissue Acid mucopolysaccharide Metabolism. Clinical Orthopaedics and Related Research, A.F. DePalma (Editor), J.B. Lippincott Co., 33:104-18, 1964.

10. Pearson, H.A., Lorincz, A.E.: A Characteristic Bone Marrow Finding in the Hurler Syndrome. Pediatrics, 34:281-82, August, 1964.

11. Brodgon, B.G., Bartley, T.D., Schiebler, G.L., Shanklin, D.R., Krovetz, L.J., Lorincz, E.E.,: Cardiovascular Radiology in Calves. Angiology, 15:496-504, November, 1964.

12. Lorincz, A.E.: Hurler's Syndrome. Medical Aspects of Mental Retardation, C.H. Carter (Editor), Chapter 19, pp. 628-650, 1965.

13. Krovetz, L.J., Lorincz, A.E., Schiebler, G.L.: Cardiovascular Manifestations in the Hurler Syndrome. Hemodynamic and Angiocardiographic Observations in 15 Patients. Circulation, 31:132-141, 1965.

14. Gennaro, J.F., Jr., Lorincz, A.E., Brewster, H.B.: The Anterior Salivary Gland of the Octopus (Octopus Vulgaris) and its Mucous Secretion. Annals of the New York Academy of Science, 118:1021-25, November, 1965.

15. Gessner, I.H., Lorincz, A.E., Bostrom, H.: Acid Mucopolysaccharide Content of the Cardiac Jelly of the Chick Embryo. Journal of Experimental Zoology, 160:291-98, No. 3, December, 1965.

16. Callahan, W.P., Lorincz, A.E.: Hepatic Ultrastructure in the Hurler Syndrome. American Journal of Pathology, 48:277-98, No. 2, February, 1966.

17. Callahan, W.P., Hacket, R.L., Lorincz, A.E.: New Observations by Light Microscopy on Liver Histology in the Hurler's Syndrome. Archives of Pathology, 83:507-12, June, 1967.

18. Lorincz, A.E.: Screening Measurements of Urinary Acid Mucopolysaccharides for Detection of the Hurler Syndrome. The Clinical Pathology of Infancy, Sunderman, F.W. & Sunderman, F.W., Jr. (Editors), pp. 68-71, 1967.

19. Rhoades, R., Lorincz, A.E., Gennaro, J.F., Jr.: Polysaccharide Content of the Poison Apparatus of the Cottonmouth Moccasin, Agkistrodon Piscivorus Piscivorus. Toxicon, 5:125-31, 1967.

BIBLIOGRAPHY (CONT'D)

20. Krovetz, L.J., Lorincz, A.E., Schiebler, G.L.: Cardiovascular Manifestations of the Hunter-Hurler Syndrome. Heart Diseases in Infants, Children and Adolescents, A.J. Moss & F.H. Adams (Editors), The Williams and Wilkins Co. (Publisher), 1968.

21. Lorincz, A.E.: Hurler's Syndrome - Disorders in Systematic Disease - Disorders of the Mucopolysaccharides. The Growth Plate and Its Disorders, M. Rang (Editor), E & S Livingston, Ltd., London, pp. 49-56, 1969.

22. Lorincz, A.E.: Pediatric Dermatology. Dermatology in General Medicine, Fitzpatrick, Thomas, B., arndt, Kenneth, A., Clark, Wallace, H., Eisen, Authur, Z., Van Scott, Eugens, J., Vaughn (Editors), John H. McGraw-Hill, Blakiston Division, N.Y., pp. 1999-2006, 1971.

23. West, S.S., Lorincz, A.E.: Fluorescent Molecular Probes in Fluorescence Microspectrophotometry and Microspectropolarimetry. Fluorescence Techniques in Cell Biology, A.A. Thaer & M. Sernetz (Editors), Springer-Verlag, Berlin-New York-Heidelburg, pp. 395-407, 1973.

24. McMahon, J., Kugel, R.B., Bartram, J.B., Berenberg, W., Cavanaugh, J.J., Hanson, V., Kennell, J.H., Lorincz, A.E., Pearson, P.H., Scott, R.B., Scurletis, T.D., Taft, L.T. (Committee on Children With Handicaps, american Academy of Pediatrics): The Physician and the Deaf child. Pediatrics, 51:1100-01, No. 6, June, 1973.

25. Thompson, J.N., Finley, S.C., Lorincz, A.E., Finley, W.H.: Absence of -L-Iduronidase Activity in Verious Tissues from Two Sibs Affected Presumably With Hurler-Scheie Compound Syndrome. Birth Defects: Original Article Series, Disorders of Connective Tissues, XI: 341-46, No. 6, 1975.

26. Hurst, R.E., Cezayirli, R.C., Lorincz, A.E.: Nature of the Glycosaminoglycanuria (Mucopolysacchariduria) in Brachycephalic "Snorter" Dwarf Cattle. Journal of Comparative Pathology, 85:481-86, 1975.

27. Lorincz, A.E., Montes, L.F.: Mucopolysaccharidosis (Type I Hurler-Scheie Compound). Journal of Cutaneous Pathology, 2:214-15, 1975.

28. Hurst, R.E., Settine, J.M., Lorincz, A.E.: A Method for the Quantitative Determinationn of Urinary Glycosaminoglycans. Clinica Chimica Acta, 70:427.32, 1976.

29. Hurst, R.E., Jennings, G.C., Lorincz, A.E.: Partition Techniques for Isolation and Fractionation or Urinary Glycosaminoglycans. Analytical Biochemistry, 79:502-12, 1977.

30. Lorincz, A.E.: The Mucoploysaccharidoses: Advances in Understanding and Treatment. Pediatric Annals, 7:64-98, 1978.

31. Lorincz, A.E.: Mucopolysaccharidoses. Medical Aspects of Mental Retardation, Charles H. Carter (Editor), Charles C. Thomas (Publisher), 2nd edition, 1978.

32. Lorincz, A.E.: Perspectives on Planning for Prevention of Mental Retardation. Planning for Services to Handicapped Persons: Community, Education, & Health, Magrab, P.R. & Elder, J.O. (Editors), P.H. Brooks (Publisher), Chapter 3, pp. 77-90, 1979.

33. Hurst, R.E., Settine, J.M., Floyd, W.M., Lorincz, A.E.: Glycosaminoglycan Excretion in Osteogenesis Imperfecta. Clinica Chimica Acta, 100:307-11, 1980.

BIBLIOGRAPHY (CONT'D)

34. Lorincz, A.E.: Learning Disabilities - A Pediatrician's Perspective. The Role of Vision in the Multidisciplinary Approach to Children with Learning Disabilities, Morton Davis & John C. Whitener (Editors), Charles C. Thomas (Publisher), Springfield, Illinois, Chapter 2, pp. 18-40, 1982.

35. Lorincz, A.E., Hurst, R.E., Kolodny, E.H.: The Early Laboratory Diagnosis of Mucopolysaccharidoses. Annals of Clinical and Laboratory Science, 12:258-66, 1982.

36. Lorincz, A.E.: Glycosaminoglycans in Heritable and Developmental Disorders of Bone. Glycosaminoglycans and Proteoglycans in Physiological and Pathological Processes of Body Systems, R.S. Varma and R. Varma (Editors), S. Karger AG (Publisher), Switzerland, pp. 264-75, 1982.

37. Lorincz, A.E., Kolodny, E.H.: The Early Diagnosis of Mucopolysaccharidoses. Proceedings of the 6th Congress of the International Association for the Scientific study of Mental Deficiency, J.M. Berg (Editor), Volume II, 1983.

38. Lorincz, A.E.: Rapid Method for the Identification of Mycoplasma Organisms. Manual of Procedures for Applications of Nucleic Acid Probes and Monoclonal Antibodies in Human Disease,
F. William Sunderman (Editor), Institute for Clinical Science, Inc. (Publisher), pp. 163-165, November 13-16, 1986.

39. Lorincz, A.E.: Rapid Fluorescence Technique for the Detection of Toxic Pulmonary Microorganisms, e.g. Legionella Pneumophila. Manual of Procedures for Clinical and Analytical Toxicology, F. William Sunderman (Editor), Institute for Clinical Science, Inc. (Publisher), pp. 129-131, November 12-15, 1987.

40. Reque, P.G., Lorincz, A.E.: Supravital Microscopic Fluorescent Technique for the Detection of Tinea capitis. Cutis, 42:111-114, August 1988.

41. Petcharuttana, Y., Cutter, G.R., Meeks, R.G., & Lorincz, A.E.: Fluorescence Microscopy of DES-induced Morphologic Transformation in Unfixed, Cultured Cells. Journal of Oral Pathology and Medicine, 18:451-456, 1989.

42. Lorincz, A.E.: Supravital Microscopic Fluorescence Technique for the Detection of Malaria and Other Parasitic Organisms. Manual of Procedures on Laboratory Diagnosis of Diseases of the Liver, F. William Sunderman (Editor), Institute for Clinical Science, Inc. (Publisher), pp. 89-92, November 16-19, 1989.

43. Lorincz, A.E.: Rapid Screening for Fungal Organisms Utilizing Supravital Flouorescence Microscopy. Manual of Procedures on Laboratory Diagnosis of Metabolic Disorders With Emphasis on Diabetes Mellitus, F. William Sunderman (Editor), Institute for Clinical Science, Inc. (Publisher), pp. 191-193, November 12-15, 1992.

44. Lorincz, A.E., Kelly, D.R., Dobbins, G.C., Cardone, V.S., Fuchs, S.A., Schilleci, J.L.: Urinalysis: Current Status and Prospects for the Future. Annals of Clinical and Laboratory Science, Vol 29, No 23,pp.169-175

ABSTRACTS

1. Lorincz, A.E., Dorfman, A.: Occurrence of Urinary Acid Mucopolysaccharides in the Hurler Syndrome. American Journal of Diseases of Children, 94:552, June, 1957.
2. Lorincz, A.E.: Acid Mucopolysaccharides in the Hurler Syndrome. Federation Proceedings, 17:226, March, 1958.
3. Kroger, M., Warnick, A.C., Hentges, J.F., Lorincz, A.E.: Mucopolysaccharidosis in Snorter Dwarf Cattle. Journal of Animal Science, 19:1221, 1960.
4. Lorincz, A.E.: "Snorter" Dwarf Cattle: A Naturally Occurring Heretable Disorder of Acid Mucopolysaccharide Metabolism Which Resembles Hurler Syndrome. American Journal of Diseases of Children, 100:488, 1960.
5. Lorincz, A.E.: Urinary Acid Mucopolysaccharides in Hereditary Deforming Chondrodysplasia (Diaphysical Aclais). Federation Proceedings, 19:148, December, 1960.
6. Lorincz, A.E.: Urinary Acid Mucopolysaccharides in Hereditary Arthrodysplasia. Southern Medical Journal, 53:1588, March, 1960.
7. Wolfson, S.L., Kahana, L., Harris, J.S., Lorincz, A.E.: Biochemical and Roetgenographic Response to Long-term Corticosteroid Therapy on the Hurler Syndrome. American Journal of Diseases of Children, 102:638, 1961.
8. Lorincz, A.E.: Mucopolysacchariduria in Children With Hereditary Arthro-osteo-onychodysplasia. Federation Proceedings, 21:173, March/April, 1962.
9. Schiebler, G.L., Lorincz, A.E., Brodgon, B.G., Shanklin, D.R., Bartley, T.D., Krovetz, L.J.: Cardiovascular Manifestations of Hurler's Syndrome. Circulation, 26:782, 1962.
10. Gessner, L.H., Lorincz, A.E.: Acid Mucopolysaccharide Content of the Three-day Old Chick Embryo Heart. Federation Proceedings, 22:2, December, 1963.
11. Krovetz, L.J., Lorincz, A.E., Schiebler, G.L.: Hemodynamic Studies in the Hunter-Hurler Syndrome (Gargoylism). Circulation, 28:753,, 1963.
12. Batey, R.L., Lorincz, A.E.: A New Technique for the Identification of Acid Mucopolysaccharide Hydrolysates. Southern Medical Journal, 56:1437, 1963.
13. Callahan, W.P., Lorincz, A.E.: Changes in Liver Ultrastructure Associated With Hurler Syndrome. Anatomical Record, 148:267, 1964.
14. Renn, W.H., Lorincz, A.E.: A New Urinary Mucopolysaccharide Associated With the Nail-Patella Syndrome (Hereditary arthro-osteo-onychodydysplasia). Southern Medical Journal, 58:1584, December, 1965.
15. Lorincz, A.E., Callahan, W.P.: Electron Microscopic Observations of Liver in the Hurler Syndrome. Southern Medical Journal, 58:1584, December, 1965.
16. Renn, W.H., Enneking, W.F., Hase, M.F., Lorincz, A.E.: Acid Mucopolysaccharide Content of Human Chondrosarcomatous Tumors. Journal of Bone and Joint Surgery, 50-A:1072, 1968.
17. Lorincz, A.E., Tiller, R.E., West, S.S.: A New Biophysical Cytochemical Finding in Viable Leukocytes Obtained from Patients with Cystic Fibrosis. Cystic Fibrosis Club Abstracts, 13th Annual Meeting, May 20, 1972.
18. Lorincz, A.E., Finley, S.C., Finley, W.H., West, S.S.: Biophysical Cytochemical Study of Glycosaminoglycans in Fibroblasts and Other Cells Derived From Marteaux-Lamy Mucopolysaccharidosis. Federation Proceedings, Vol. 31, No. 2, March-April, 1973.

ABSTRACTS (CONT'D)

19. Warren, R.J., Condron, C.J., Hollister, D., Huifing, F. Neufeld, E.F., Hall, C.W., Mcleod, A.G.W., Lorincz, A.E.: Antenatal Diagnosis of Mucolipiddosis II (I-Cell Disease). Pediatric Research, Vol. 7, No. 343, April, 1973.
20. Lorincz, A.E., West, S.S.: The Biophysical Cytochemistry of Living Cells Derived From Patients With Mucopolysaccharidoses. Journal of Bone and Joint Surgery; Proceedings of the 19th Annual Meeting of the Orthopedic Research Society, Las Vegas, Nevada, 55-A:655, January 30, 31 & February 1, 1973.
21. Hurst, R.E., Cezayirli, R.C., Lorincz, A.E.: The Nature of the Glycosaminoglycans (Mucoploysacchariduria) in Brachycephalic "Snorter" Dwarf Cattle. Federation Proceedings, 34:861, March, 1975.
22. Lorincz, A.E., Hurst, R.E., Cezayirli, R.C.: Definitive Characterization of the Glycosaminoglycanuria in "Snorter" Dwarf Cattle. Pediatric Research, 9:314, 1975.
23. Lorincz, A.E., Hurst, R.E., Floyd, W.M.: Glycosaminoglycan excretion of Osteogenesis Imperfecta. Transactions of the 25th Annual Meeting. Orthopedic Research Society; Journal of Bone and Joint Surgery, 4:108, 1978.
24. Lorincz, A.E.: Clinical Chemistry in the Profoundly Retarded. Annals of Clinical and Laboratory Science, 14:316-17, 1984.
25. Lorincz, A.E.: Direct Visualization of Mycoplasma via Supravital Staining and Fluorescence Microscopy. Abstracts of the 6th International Congress of the International Organization for Mycoplasma, p. 272, 1986.
26. Lorincz, A.E., Thompson, J.N.: Use of Microscopic Fluorescence Amniotic Fluid to Diagnose Intrauterine Mucopolysaccharidosis. 1st International Congress on Mucopolysaccharidosis and Related Diseases, p. 33, 1988.
27. Lorincz, A.E., Reque, P.G.. Comparison of Supravital Microscopic Fluorescence Technique (SMFT) to Darkfield Microscopy for Detection of Spirochetes. Annals of Clinical and Laboratory Science, 20:281-82, 1990.
28. Lorincz, A.E.: One Step On-site Epi-fluorescence Detection of Fungi: A Possible Alternate to KOH Screening. Annals of Clinical and Laboratory Science, 23: 307, 1993.
29. Lorincz, A.E. and Bueschen, A.J.: Mycoplasma-like Mollicutes in Human Prostate Tumor Tissue. Annals of Clinical and Laboratory Science, 24: 196, 1994.
30. Lorincz, A.E., Baltaro, R.J. and Adamson, D.M.: Detection of Significant Bacteruria Using Supravital Fluorescence Microscopy. Annals of Clinical and Laboratory Science, 25: 363, 1995.
31. Lorincz, A.E., Bueschen, A.J., and Urban, D.A.: Presence of Mycoplasma-Like Mollicutes In Prostate Needle Biopsy Specimens, Annals of Clinical and Laboratory Science, 26:364, 1996.

EDITORIALS AND COMMENTARIES

1. Lorincz, A.E.: Biochemical Genetic Defects. Journal of the Florida Medical Association, 66:852, 1960.
2. Lorincz, A.E., Smith, R.T.: Research at the University of Florida College of Medicine, Department of Pediatrics. Journal of the Florida Medical Association, 51:808-11, 1965.
3. Lorincz, A.E.: Birth Defects - A Continuing Challenge. Journal of the Florida Medical Association, 55:126-27, 1968.

BOOK REVIEWS

1. "Modern Topics in Paediatric Dermatology", Verbov, J. (Editor), J.B. Lippincott, Philadelphia (Publisher), JAMA, 244:1269, 1980.
2. "Dermatologic Disorders in Black Children and Adolescents", Laude, T.A. & Russo, R.U. (Editors), Medical Examination Publishing Company, New Hyde Park, New York (Publisher), JAMA, 252:1769-70, 1984.
3. "Practical Pediatric Dermatology", Weston, W.L. (Editor), Little Brown and Conpany, Boston (Publisher), JAMA, 277:549, 1987.

EXHIBIT
B

DISCLOSURES

APPLICATION
SERIAL NO. 09/836,750

EXHIBIT B

DISCLOSURES
APPLICATION SERIAL NO. 09/836,750

PAGE 20, LINE 10 – PAGE 21, LINE 15

Growth factors can be utilized to induce the growth of "hard tissue" or bone and "soft tissues" like ectodermal and mesodermal tissues. As used herein, the term growth factor encompasses compositions and living organisms which promote the growth of hard tissue, such as bone, or soft tissue, in the body of a patient. The compositions include organic and inorganic matter. The compositions can be genetically produced or manipulated. The living organisms can be bacteria, viruses, or any other living organism which promote tissue growth. By way of example and not limitation, growth factors can include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (acidic/basis (FGF a,b), interleukins (IL's), tumor necrosis factor (TNF), transforming growth factor (TGF-B), colony-stimulating factor (CSF), osteopontin (Eta-1 OPN), platelet-derived growth factor (PDGF), interferon (INF), bone morphogenic protein 1 (BMP-1), and insulin growth factor (IGF). Recombinant and non-recombinant growth factors can be utilized as desired. Bacteria or viruses can, when appropriate, be utilized as growth factors. For example, there is a bacterial hydrophilic polypeptide that self-assembles into a nanometer internal diameter pore to build a selective lipid body. Various enzymes can be utilized for the synthesis of peptides which contain amino acids that control three-dimensional protein structure and growth. Growth factors can be applied in gels or other carriers which regulate the rate of release of the growth factors and help maintain the growth factors and the carrier, at a desired location in the body. Time release capsules, granules, or other carriers containing growth factor can be activated by tissue pH, by enzymes, by ultrasound, by electricity, by heat, by selected *in vivo* chemicals or by any other selected means to release the growth factor. The carrier can be resorbable or non-resorbable. Or, the growth factor itself can be activated by similar means. Either the carrier or the growth factor can mimic extracellular fluid to control cell growth, migration, and function. The growth factor can be administered orally, systemically, in a carrier, by hypodermic needle, through the respiratory tract, or by any other desired method. The growth factor an also be administered into a capsule or other man-made composition or structure placed in the body. While administration of the growth factor is presently usually localized in the patient's body, circumstances may arise where it is advantageous to distribute a growth factor throughout the patient's body in uniform or non-uniform concentrations. An advantage to growth factors is that they can often, especially when in capsule form or in some other containment system, be inserted to a desired site in the body by simply making a small incision and inserting the growth factor. The making of such small incision comprises minor surgery which an often be accomplished on an out-patient basis. The growth factors can be multifactorial and nonspecific.

PAGE 44, LINE 19 – PAGE 46, LINE 16

Genetic material comprising a portion of a gene, a gene, genes, a gene product (i.e., a composition a gene causes to be produced like, for example, an organ-producing growth factor), growth factor, or an ECM (extracellular matrix) can be used in or on the body to grow an organ to tissue. For example, the vascular epithelial growth factor gene (VEGF) or its growth factor equivalent can be inserted into the body to cause an artery to grow. When insertion of a gene, portion of a gene, gene product, growth factor, or ECM *in vivo* or *ex vivo* is referred to herein in connection with any of the implant techniques of the invention, it is understood that a cell nutrient culture(s), physiological nutrient culture(s), carrier (s), enhancer(s), promoter(s), or any other desired auxiliary component(s) can be inserted with the gene or at the same location as the gene, growth factor, ECM, etc.

An artery is an organ from the circulatory system. An artery can be grown in the heart, legs, or other areas by injecting a gene or other genetic material into muscle at a desired site. Size, vascularity, simplicity of access, ease of exploitation, and any other desired factors can be utilized in selecting a desired site. The gene is one of several known VEGF genes which cause the production of vascular endothelial growth factors. Several VEGF genes which produce vascular endothelial growth factors are believed to exist because nature intends for there to be several pathways (i.e., genes) which enable the production of necessary growth factors. The existence of several pathways is believed important because if one of the genes is damaged or inoperative, other similar genes can still orchestrate the production of necessary growth factors. VEGF genes are used by the body to promote blood vessel growth. VEGF genes are assimilated (taken in) by muscle cells. The genes cause the muscle cells to make a VEGF protein which promotes the growth of new arteries. VEGF proteins can be made in a lab and injected into a patient intravenously, intraluminally, or intramuscularly to promote the growth of an artery. Or, the genes (or other genetic material) can be applied with an angioplasty balloon, with the assistance of a vector, or by any other method.

It is not always desirable to grow a completely new organ. Sometimes growing a portion of an organ is desirable. For example, in some heart attacks or strokes, a portion of the heart or brain remains viable and a portion dies. An injection of a gene to form cardiac muscle and/or an injection of a gene to form an artery can be utilized to revive or replace the dead portion of the heart. The dead portion of the heart may (or may not) be used as a matrix while the new muscles and vessels grow. Thus, in this example, a partial new organ is grown in a pre-existing organ. A pacemaker may (or may not) be necessary. A second injection of a gene may (or may not) be necessary to stop cardiac muscle growth once it is completed. Portions of organs throughout the body can similarly be repaired or replaced. It may be necessary to provide gene(s) or growth factor(s) sequentially. For instance, one or more blood vessels are grown by inserting an appropriate gene or other genetic material into a selected area. Second, an appropriate gene or other genetic material is inserted in the selected area to grow a bone or other organ.

The size and shape limitation of the desired structure can come from a containment and boundary contact inhibition phenomenon or by a chemical inhibition.

A variation on the theme of growing a portion of an organ is as follows: a portion of a heart dies. The pericardium is utilized as a scaffold and seeded with cells and/or genes to grow new muscle, and genes (or other genetic material) to grow new arteries. Immediately adjacent the dead cardiac muscle, onto or into the pericardium, the appropriate cells, genes, and/or growth factors (or other genetic material) are placed. Once the new muscle and blood vessels have grown, the function specific tissue can be applied to the damaged portion of the heart and paced, if necessary, to augment cardiac action. If the surgeon desires, the dead muscle can be removed and the new muscle and blood vessels can be surgically rotated into the excised region and secured. This probably can be done endoscopically. In essence, the pericardium is utilized to allow the new muscle wall to grow. The new muscle wall is then transplanted into the damaged heart wall. This procedure utilizes the body as a factor to grow an organ and/or tissue, after which the organ and/or tissue is transplanted to a desired region. On the other hand, the new muscle wall may integrate itself into the old wall and not require transplantation.

EXHIBIT C

DEFINITIONS

EXHIBIT C

DEFINITIONS

PAGE 20, LINE 10 – PAGE 21, LINE 15

Growth factors can be utilized to induce the growth of "hard tissue" or bone and "soft tissues" like ectodermal and mesodermal tissues. As used herein, the term growth factor encompasses compositions and living organisms which promote the growth of hard tissue, such as bone, or soft tissue, in the body of a patient. The compositions include organic and inorganic matter. The compositions can be genetically produced or manipulated. The living organisms can be bacteria, viruses, or any other living organism which promote tissue growth. By way of example and not limitation, growth factors can include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (acidic/basis (FGF a,b), interleukins (IL's), tumor necrosis factor (TNF), transforming growth factor (TGF-B), colony-stimulating factor (CSF), osteopontin (Eta-1 OPN), platelet-derived growth factor (PDGF), interferon (INF), bone morphogenic protein 1 (BMP-1), and insulin growth factor (IGF). Recombinant and non-recombinant growth factors can be utilized as desired. Bacteria or viruses can, when appropriate, be utilized as growth factors. For example, there is a bacterial hydrophilic polypeptide that self-assembles into a nanometer internal diameter pore to build a selective lipid body. Various enzymes can be utilized for the synthesis of peptides which contain amino acids that control three-dimensional protein structure and growth. Growth factors can be applied in gels or other carriers which regulate the rate of release of the growth factors and help maintain the growth factors and the carrier, at a desired location in the body. Time release capsules, granules, or other carriers containing growth factor can be activated by tissue pH, by enzymes, by ultrasound, by electricity, by heat, by selected *in vivo* chemicals or by any other selected means to release the growth factor. The carrier can be resorbable or non-resorbable. Or, the growth factor itself can be activated by similar means. Either the carrier or the growth factor can mimic extracellular fluid to control cell growth, migration, and function. The growth factor can be administered orally, systemically, in a carrier, by hypodermic needle, through the respiratory tract, or by any other desired method. The growth factor an also be administered into a capsule or other man-made composition or structure placed in the body. While administration of the growth factor is presently usually localized in the patient's body, circumstances may arise where it is advantageous to distribute a growth factor throughout the patient's body in uniform or non-uniform concentrations. An advantage to growth factors is that they can often, especially when in capsule form or in some other containment system, be inserted to a desired site in the body by simply making a small incision and inserting the growth factor. The making of such small incision comprises minor surgery which an often be accomplished on an out-patient basis. The growth factors can be multifactorial and nonspecific.

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Growth factor: a substance (as a vitamin B_{12} or an interleukin) that promotes growth and especially cellular growth

EXHIBIT D

CLAIMS

EXHIBIT D

CLAIMS

Claim X: A method for growing a new portion of a pre-existing heart comprising the steps of: placing a growth factor in a body of a human patient and growing new muscle in said heart.

EXHIBIT E

PUBLICATIONS

EXHIBIT E

PUBLICATION INFORMATION SUMMARY

TITLE	AUTHOR	CITATION	DATE	AUTHOR COUNTRY	ROUTE OF ADMINISTRATION	GROWTH FACTOR ADMINISTERED	RESULT
Left Ventricular Electromechanical Mapping to Assess Efficacy of phVEGF165 Gene Transfer for Therapeutic Angiogenesis in Chronic Myocardial Ischemia	Vale	Circulation. 2000; 102:965-974	08/29/00	U.S.	Small incision (minithoracotomy) with syringe injection	VEGF (Gene form)	Repair of damaged portion of heart – Also pertains to new muscle growth
Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans	Strauer	Circulation. 2002; 106:1913-1918	10/08/02	Germany	Balloon catheter with injection	Bone Marrow Cells	Repair of dead portion of heart – also pertains to new muscle growth

TITLE	AUTHOR	CITATION	DATE	AUTHOR COUNTRY	ROUTE OF ADMINISTRATION	GROWTH FACTOR ADMINISTERED	RESULT
Viability and differentiation of autologous skeletal myoblast grafts in ischemic cardiomyopathy	Hagege	Lancet 2003 Feb 8; 361 (9356):491-492	2003	France	Injection	Skeletal Muscle Cells	Repair of dead portion of heart; Histological Proof (muscle)
Autologous Cell Transplant Helpful in Ischemic Heart or Legs	Barclay	Medscape Medical News 2000 – Abstract from American Heart Association's 75th Scientific Sessions on 11/18/02, Chicago	11/18/02	U.S.	Surgery with syringe injection	Bone Marrow Cells	Repair of damaged portion of heart – also pertains to new muscle growth
Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation	Pagani	J Am Coll Cardiol 2003 Mar 5; 41(5): 879-888	2003	U.S.	Surgery with syringe injection	Skeletal Muscle Cells	Repair of dead portion of heart; Histological Proof (muscle and blood vessels)

Clinical Investigation and Reports

Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans

Bodo E. Strauer, MD; Michael Brehm, MD; Tobias Zeus, MD; Matthias Köstering, MD;
Anna Hernandez, PhD; Rüdiger V. Sorg, PhD; Gesine Kögler, PhD; Peter Wernet, MD

Background—Experimental data suggest that bone marrow–derived cells may contribute to the healing of myocardial infarction (MI). For this reason, we analyzed 10 patients who were treated by intracoronary transplantation of autologous, mononuclear bone marrow cells (BMCs) in addition to standard therapy after MI.

Methods and Results—After standard therapy for acute MI, 10 patients were transplanted with autologous mononuclear BMCs via a balloon catheter placed into the infarct-related artery during balloon dilatation (percutaneous transluminal coronary angioplasty). Another 10 patients with acute MI were treated by standard therapy alone. After 3 months of follow-up, the infarct region (determined by left ventriculography) had decreased significantly within the cell therapy group (from 30±13 to 12±7%, $P=0.005$) and was also significantly smaller compared with the standard therapy group ($P=0.04$). Likewise, infarction wall movement velocity increased significantly only in the cell therapy group (from 2.0±1.1 to 4.0±2.6 cm/s, $P=0.028$). Further cardiac examinations (dobutamine stress echocardiography, radionuclide ventriculography, and catheterization of the right heart) were performed for the cell therapy group and showed significant improvement in stroke volume index, left ventricular end-systolic volume and contractility (ratio of systolic pressure and end-systolic volume), and myocardial perfusion of the infarct region.

Conclusions—These results demonstrate for the first time that selective intracoronary transplantation of autologous, mononuclear BMCs is safe and seems to be effective under clinical conditions. The marked therapeutic effect may be attributed to BMC-associated myocardial regeneration and neovascularization. (***Circulation.*** **2002;106:1913-1918.**)

Key Words: myocardial infarction ■ cell transplantation, intracoronary ■ angiogenesis ■ bone marrow ■ myogenesis

Remodeling of the left ventricle after myocardial infarction (MI) represents a major cause of infarct-related heart failure and death. This process depends on acute and chronic transformation of both the necrotic infarct region and the non-necrotic, peri-infarct tissue.[1,2] Despite application of pharmacotherapeutics and mechanical interventions, the cardiomyocytes lost during MI cannot be regenerated. The recent finding that a small population of cardiac muscle cells is able to replicate itself is encouraging but is still consistent with the concept that such regeneration is restricted to viable myocardium.[3]

In animal experiments, attempts to replace the necrotic zone by transplanting other cells (eg, fetal cardiomyocytes or skeletal myoblasts) have invariably succeeded in reconstituting heart muscle structures, ie, myocardium and coronary vessels. However, these cells fail to integrate structurally and do not display characteristic physiological functions.[4–7] Another approach to reverse myocardial remodeling is to repair myocardial tissue by using bone marrow–derived cells. Bone marrow contains multipotent adult stem cells that show a high capacity for differentiation.[8–10] Experimental studies have shown that bone marrow cells (BMCs) are capable of regenerating infarcted myocardium and inducing myogenesis and angiogenesis; this leads in turn to amelioration of cardiac function in mice and pigs.[11–14] However, procedures based on this phenomenon remain largely uninvestigated in a human clinical setting.

An investigation of one patient receiving autologous skeletal myoblasts into a postinfarction scar during coronary artery bypass grafting revealed improvement of contraction and viability 5 months afterward.[15] Autologous mononuclear BMCs transplanted in a similar surgical setting showed long-term improvement of myocardial perfusion in 3 of 5 patients and no change in 2 patients.[16] However, such studies entail a surgical approach and are therefore associated with well-known perioperative risks. Moreover, this surgical procedure cannot be used with MI. We therefore looked for a nonsurgical, safer mode for transplanting autologous cells

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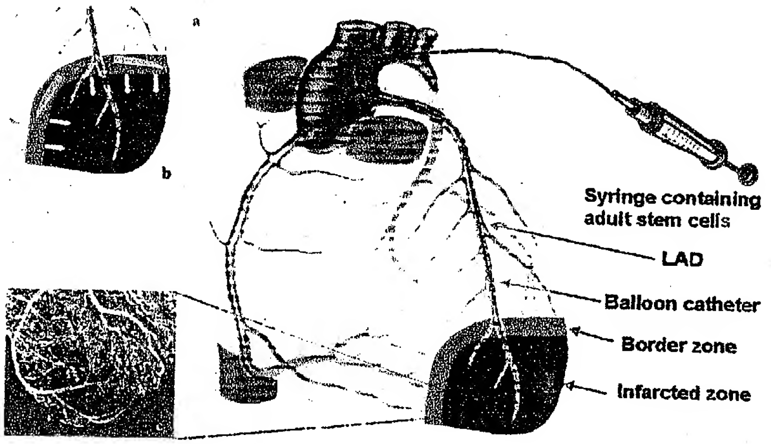

Figure 1. Procedure of cell transplantation into infarcted myocardium in humans. a, The balloon catheter enters the infarct-related artery and is placed above the border zone of the infarction. It is then inflated and the cell suspension is infused at high pressure under stop-flow conditions. b, In this way, cells are transplanted into the infarcted zone via the infarct-related vasculature (red dots). Cells infiltrate the infarcted zone. Blue and white arrows suggest the possible route of migration. c, A supply of blood flow exists within the infarcted zone.[35] The cells are therefore able to reach both the border and the infarcted zone.

into postinfarction tissue. A pilot study from our group demonstrated that intracoronary transplantation of autologous mononuclear BMCs 6 days after MI was associated with a marked decrease in infarct area and an increase in left ventricular (LV) function after 3 and 6 months of follow-up.[17] To confirm these results and validate this promising new therapy for MI, we established a clinical trial involving 20 patients for comparing the safety and bioefficacy of autologous BMC transplantation. All 20 patients underwent standard therapy, and 10 patients received additional intracoronary cell transplantation. All 20 patients were followed up for 3 months.

Methods

Patient Population

All 20 patients had suffered transmural infarction according to World Health Organization criteria with the involvement of the left anterior descending coronary artery (n=4), left circumflex coronary artery (n=3), or right coronary artery (n=13). Mean duration of infarct pain was 12±10 hours before invasive diagnostics and therapy. Patients had to be <70 years old and were excluded if one of the following criteria were met: screening >72 hours after infarction, cardiac shock, severe comorbidity, alcohol or drug dependency, or excessive travel distance to the study center.

After right and left heart catheterization, coronary angiography, and left ventriculography, mechanical treatment was initiated with recanalization of the infarct-related artery by balloon angioplasty (n=20) and subsequent stent implantation (n=19). All patients were monitored in our intensive care unit, and no arrhythmogenic events or hemodynamic impairments were recorded in either patient group.

All 20 patients were briefed in detail about the procedure of BMC transplantation. Informed consent was obtained from 10 patients, who formed the cell therapy group, whereas 10 patients who refused additional cell therapy served as controls. The local ethics committee of the Heinrich-Heine-University, Düsseldorf, approved the study protocol. All procedures conformed to institutional guidelines.

Before taking part in rehabilitation programs, all patients left the hospital with standard medication consisting of acetylsalicylic acid, an ACE inhibitor, a β-blocker, and a statin.

Bone Marrow Aspiration, Isolation, and Cultivation

Seven (±2) days after acute coronary angiography, bone marrow (~40 mL) was aspirated under local anesthesia from ilium of cell therapy patients (n=10). Mononuclear BMCs were isolated by Ficoll density separation on Lymphocyte Separation Medium (BioWhittaker) before the erythrocytes were lysed with H_2O. For overnight cultivation, 1×10^6 BMCs/mL were placed in Teflon bags (Vuelife, Cell Genix) and cultivated in X-Vivo 15 Medium (BioWhittaker) supplemented with 2% heat-inactivated autologous plasma. The next day, BMCs were harvested and washed 3 times with heparinized saline before final resuspension in heparinized saline. Viability was 93±3%. Heparinization and filtration (cell strainer, FALCON) was carried out to prevent cell clotting and microembolization during intracoronary transplantation. The mean number of mononuclear cells harvested after overnight culture was 2.8×10^7; this consisted of 0.65±0.4% AC133-positive cells and 2.1±0.28% CD34-positive cells. All microbiological tests of the clinically used cell preparations proved negative. As a viability and quality ex vivo control, 1×10^5 cells grown in H5100 medium (Stem Cell Technology) were found to be able to generate mesenchymal cells in culture.

Intracoronary Transplantation of BMCs

Five to nine days after onset of acute infarction, cells were directly transplanted into the infarcted zone (Figure 1). This was accomplished with the use of a balloon catheter, which was placed within the infarct-related artery. After exact positioning of the balloon at the site of the former infarct-vessel occlusion, percutaneous transluminal coronary angioplasty (PTCA) was performed 6 to 7 times for 2 to 4 minutes each. During this time, intracoronary cell transplantation via the balloon catheter was performed, using 6 to 7 fractional high-pressure infusions of 2 to 3 mL cell suspension, each of which contained 1.5 to 4×10^6 mononuclear cells. PTCA thoroughly prevented the backflow of cells and at the same time produced a stop-flow beyond the site of the balloon inflation to facilitate high-pressure infusion of cells into the infarcted zone. Thus, prolonged contact time for cellular migration was allowed.[18]

Functional Assessment of Hemodynamics

After 3 months, all 20 patients were followed up by left heart catheterization, left ventriculography, and coronary angiography. Ejection fraction, infarct region, and regional wall movement of the infarcted zone during ejection were determined by left ventriculography. Ejection fraction was measured with Quantcor software (Siemens). To quantify infarction wall movement velocity, 5 axes were placed perpendicular to the long axis in the main akinetic or dyskinetic segment of the ventricular wall. Relative systolic and diastolic lengths were measured, and the mean difference was divided by the systolic duration (in seconds). To quantify the infarct region, the centerline method according to Sheehan was used.[19] All hemodynamic investigations were obtained by two independent observers.

In the cell therapy group before and 3 months after cell transplantation, additional examinations for measuring hemodynamics and myocardial perfusion included dobutamine stress echocardiography, radionuclide ventriculography, catheterization of the right heart, and

TABLE 1. Baseline Characteristics of the Patients

Clinical Data	Cell Therapy	Standard Therapy	*P*
Characteristics			
No. of patients	10	10	. . .
Age, y	49±10	50±6	NS
Sex	Male	Male	. . .
Onset of infarction before angioplasty, h	10±8	13±11	NS
Coronary angiography			
No. of diseased vessels	1.7±0.9	2.1±0.7	NS
No. of patients with LAD/LCX/RCA as the affected vessel	4/1/5	0/2/8	. . .
No. of patients with stent implantation	9	10	. . .
Laboratory parameters			
Creatinine kinase, U/L	1138±1170	1308±1187	NS
Creatinine kinase-MB, U/L	106±72	124±92	NS
Bone marrow puncture after angioplasty, d	7±2	. . .	. . .
Mononuclear bone marrow cells, n ($\times 10^7$)	2.8±2.2	. . .	. . .

Values are mean±SD or number of patients.

NS indicates not significant; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; and RCA, right coronary artery.

stress-redistribution-reinjection ^{201}thallium scintigraphy. The contractility index P_{syst}/ESV was calculated by dividing LV systolic pressure (P_{syst}) by end-systolic volume (ESV). Perfusion defect was calculated by scintigraphic bull's-eye technique. Each examination was performed according to standard protocols.

There were no complications or side effects determined in any patient throughout the diagnostic or therapeutic procedure or within the 3-month follow-up period.

Statistical Analysis

All data are presented as mean±SD. Statistical significance was accepted when *P* was <0.05. Discrete variables were compared as rates, and comparisons were made by χ^2 analysis. Intra-individual comparison of baseline versus follow-up continuous variables was performed with a paired *t* test. Comparison of nonparametric data between the two groups was performed with Wilcoxon test and Mann-Whitney test. Statistical analysis was performed with SPSS for Windows (version 10.1).

Results

Clinical data between the two groups did not differ significantly. The range of creatinine kinase levels was slightly but not significantly higher in the standard therapy group than it was in the cell therapy group (Table 1).

Comparison of the 2 groups 3 months after cell or standard therapy showed several significant differences in LV dynamics, according to the global and regional analysis of left ventriculogram. The infarct region as a percentage of hypokinetic, akinetic, or dyskinetic segments of the circumference of the left ventricle decreased significantly in the cell therapy group (from 30±13 to 12±7%, *P*=0.005). It was also significantly smaller compared with the standard therapy group after 3 months (*P*=0.04). Within the standard therapy group, only a statistically nonsignificant decrease from 25±8 to 20±11% could be seen. Wall movement velocity over the infarct region rose significantly in the cell therapy group (from 2.0±1.1 to 4.0±2.6 cm/s, *P*=0.028) but not in the standard therapy group (from 1.8±1.3 to 2.3±1.6 cm/s, *P*=NS). No significant difference was observed between the two groups. Ejection fraction increased in both groups, albeit nonsignificantly (from 57±8 to 62±10% in the cell therapy group and from 60±7 to 64±7% in the standard therapy group) (Table 2).

Further significant improvement could also be seen on additional analysis of the cell therapy group alone. Perfusion defect was considerably decreased by 26% in the cell therapy group (from 174±99 to 128±71 cm^2, *P*=0.016, assessed by ^{201}thallium scintigraphy) (Figure 2). Parallel to the reduction in perfusion defect, improvement (Table 3) could also be seen in:

(1) Cardiac function, as revealed by increase in stroke volume index (from 49±7 to 56±7 mL/m^2, *P*=0.010) and ejection fraction (from 51±14 to 53±13%, *P*=NS).
(2) Cardiac geometry, as shown by decreases in both end-diastolic (from 158±20 to 143±30 mL, *P*=NS) and end-systolic volume (from 82±26 to 67±21 mL, *P*=0.011). Radionuclide ventriculography was used to acquire the data.
(3) Contractility as evaluated by an increase in the velocity of circumferential fiber shortening (from 20.5±4.2 to 24.4±7.7 mm/s, *P*=NS, assessed by stress echocardiography) and by a marked increase in the ratio of systolic pressure to end-systolic volume (from 1.81±1.44 to 2.27±1.72 mm Hg/mL, *P*=0.005).

Discussion

The present report describes the first clinical trial of intracoronary, autologous, mononuclear BMC transplantation for improving heart function and myocardial perfusion in patients after acute MI. The results demonstrate that transplanted autologous BMCs may lead to repair of infarcted tissue when applied during the immediate postinfarction period. These results also show that the intracoronary approach of BMC transplantation seems to represent a novel

TABLE 2. Comparison of Cell Therapy and Standard Therapy Groups

	Cell Therapy	Standard Therapy	*P*
No. of patients	10	10	...
Infarct region as functional defect			
Hypokinetic, akinetic, or dyskinetic region at 0 mo, %	30±13	25±8	NS
Hypokinetic, akinetic, or dyskinetic region at 3 mo, %	12±7	20±11	0.04
P	0.005	NS	...
Contractility indices			
Infarction wall movement velocity at 0 mo, cm/s	2.0±1.1	1.8±1.3	NS
Infarction wall movement velocity at 3 mo, cm/s	4.0±2.6	2.3±1.6	NS
P	0.028	NS	...
Hemodynamic data			
LV ejection fraction at 0 mo, %	57±8	60±7	NS
LV ejection fraction at 3 mo, %	62±10	64±7	NS
P	NS	NS	...

NS indicates not significant; 0 mo, zero months, which means the time of infarction; 3 mo, 3 months, which means the time of the follow-up examinations. All data were obtained according to analysis of left ventriculogram.

and effective therapeutic procedure for concentrating and/or depositing infused cells within the region of interest.

Neogenesis of both cardiomyocytes and coronary capillaries with some functional improvement has been shown recently by several investigators using bone marrow–derived cells in experimental infarction.[11–14,18,20–23] Moreover, transendothelial migration from the coronary capillaries and incorporation of cells into heart muscle has been observed experimentally.[3,12,24–26] Until now, clinical data only existed for the cell therapy of surgically treated chronic ischemic heart disease.[15,16] Our aim was to transform the encouraging results from animal models to a safe clinical setting. The most crucial questions we had to address while designing and realizing this trial were: (1) What cell population should we deliver? (2) Which application method is the most efficient? (3) When should the cells be transplanted?

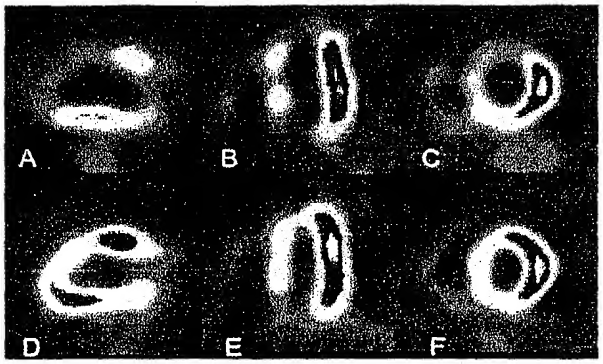

Figure 2. Improved myocardial perfusion of infarcted anterior wall 3 months after intracoronary cell transplantation subsequent to an acute anterior wall infarction detected by ^{201}thallium scintigraphy. The images on the left (A, D, sagittal) and in the middle (B, E) show the long axis, whereas those on the right (C, F, frontal) show the short axis of the heart. Initially the anterior wall, with green-colored apical and anterior regions, had reduced myocardial perfusion (A, B, C). Three months after cell transplantation the same anterior wall, now yellow in color, revealed a significant improvement in myocardial perfusion (D, E, F). All illustrations depict the exercise phase.

In recent years, several laboratories have shown that environmentally dictated changes of fate (transdetermination) are not restricted to stem cells but may also involve progenitor cells at different steps of a given differentiation pathway (transdifferentiation). Moreover, mesenchymal stem cells may represent an ideal cell source for treating different diseases.[27] Adult, mononuclear BMCs contain such stem and progenitor cells (≤1%), eg, mesodermal progenitor cells, hematopoietic progenitor cells, and endothelial progenitor cells. In several animal infarction models it has been shown that: (1) Bone marrow hemangioblasts contribute to the formation of new vessels; (2) bone marrow hematopoietic stem cells differentiate into cardiomyocytes, endothelium,

TABLE 3. Cardiac Function Analysis at 3-Month Follow-Up

	Before Cell Therapy	3 Months After Cell Therapy	*P*
No. of patients	10	10	...
Hemodynamic data			
LV ejection fraction, %	51±14	53±13	NS
Stroke volume index, mL/m^2	49±7	56±7	0.010
Cardiac geometry			
LV end-diastolic volume, mL	158±20	143±30	NS
LV end-systolic volume, mL	82±26	67±21	0.011
Contractility indices			
Circumferential fiber shortening, mm/s	20.5±4.2	24.4±7.7	NS
P_{sys}/ESV, mm Hg/mL	1.81±1.44	2.27±1.72	0.005
Infarct region as perfusion defect			
^{201}Thallium scintigraphy, cm^2	174±99	128±71	0.016

NS indicates not significant.

and smooth muscle cells[8-13]; (3) BMCs give rise to mesodermal progenitor cells that differentiate to endothelial cells[28]; and (4) endothelial progenitors can transdifferentiate into beating cardiomyocytes.[29] Thus, several different fractions of mononuclear BMCs may contribute to the regeneration of necrotic myocardium and vessels. In order to utilize this large and perhaps heterogeneous regenerative potential, we decided to use all mononuclear cells from the bone marrow aspirate as a whole, rather than a subpopulation. No further expansion was performed because experimental data have revealed a dramatic decline in the homing capacity of in vitro amplified hematopoietic stem or progenitor cells.[30]

The second question was how to deliver the cells most efficiently. When given intravenously, only a very small fraction of infused cells can reach the infarct region after the following injection: assuming a normal coronary blood flow of 80 mL/min per 100 g of LV weight, a quantity of 160 mL per left ventricle (assuming a regular LV mass of ≈200 g) will flow per minute.[31,32] This corresponds to only about 3% of cardiac output (assuming a cardiac output of 5000 mL/min).[31] Therefore, intravenous application would require many circulation passages to enable infused cells to come into contact with the infarct-related artery. Throughout this long circulation and recirculation time, homing of cells to other organs could considerably reduce the numbers of cells dedicated to cell repair in the infarcted zone. Thus, supplying the entire complement of cells by intracoronary administration obviously seems to be advantageous for the tissue repair of infarcted heart muscle and may also be superior to intraventricular injection,[33] because all cells are able to flow through the infarcted and peri-infarcted tissue during the immediate first passage. Accordingly, by this intracoronary procedure the infarct tissue and the peri-infarct zone can be enriched with the maximum available amount of cells at all times.

As stem cells differentiate into more mature types of progenitor cells, it is thought that a special microenvironment in so-called niches regulates cell activity by providing specific combinations of cytokines and by establishing direct cellular contact. For successful long-term engraftment, at least some stem cells have to reach their niches, a process referred to as homing. Mouse experiments have shown that significant numbers of BMCs appear in liver, spleen, and bone marrow after intravenous injection.[34] To offer the BMCs the best chance of finding their niche within the myocardium, a selective intracoronary delivery route was chosen. Presumably, therefore, fewer cells were lost by extraction toward organs of secondary interest by this first pass–like effect. To facilitate transendothelial passage and migration into the infarcted zone, cells were infused by high-pressure injection directly into the necrotic area, and the balloon was kept inflated for 2 to 3 minutes; the cells were not washed away immediately under these conditions.

The time point for delivery was chosen as 7 to 8 days after infarction onset for the following reasons:

(1) In dogs, infarcted territory becomes rich in capillaries and contains enlarged, pericyte-poor "mother vessels" and endothelial bridges 7 days after myocardial ischemia and reperfusion. Twenty-eight days later, a significant muscular vessel wall has already formed.[35] Thus, with such timing, cells may be able to reach the worst damaged parts and at the same time salvage tissue. Transendothelial cell migration may also be enhanced because an adequate muscular coat is not yet formed.

(2) Until now, only one animal study has attempted to determine the optimum time for cardiomyocyte transplantation to maximize myocardial function after LV injury. Adult rat hearts were cryoinjured and fetal rat cardiomyocytes were transplanted immediately, 2 weeks later, and 4 weeks later. The authors discussed the inflammatory process, which is strongest in the first days after infarction, as being responsible for the negative results after immediate cell transplantation, and they assumed that the best results seen after 2 weeks may have been due to transplantation before scar expansion.[36] Until now, however, no systematic experiments have been performed with BMCs to correlate the results of transplantation with the length of such a time delay.

(3) Another important variable is the inflammatory response in MI, which seems to be a superbly orchestrated interaction of cells, cytokines, growth factors and extracellular matrix proteins mediating myocardial repair. In the first 48 hours, debridement and formation of a fibrin-based provisional matrix predominates before a healing phase ensues.[37-40] Moreover, vascular endothelial growth factor is at its peak concentration 7 days after MI, and the decline of adhesion molecules (intercellular adhesion molecules, vascular cell adhesion molecules) does not take place before days 3 to 4 after MI. We assumed that transplantation of mononuclear BMCs within the "hot" phase of post-MI inflammation might lead them to take part in the inflammation cascade rather than the formation of functional myocardium and vessels.

Taking all of this into account, we can conclude that cell transplantation within the first 5 days after acute infarction is not possible for logistical reasons and is not advisable because of the inflammatory process. On the other hand, transplantation 2 weeks after infarction scar formation seems to reduce the benefit of cell transplantation. Although the ideal time point for transplantation remains to be defined, it is most likely between days 7 and 14 after the onset of MI, as in the present study.

This trial was designed as a phase I safety and feasibility trial, meaning that no control group is necessarily required. However, to validate the results, we correlated them with those obtained from 10 patients who refused to get additional cell therapy and thus received standard therapy alone. We are aware of the fact that such a comparison does not reach the power of a randomly allocated, blinded control group. However, the significant improvement with regard to infarct region, hemodynamics (stroke volume index), cardiac geometry (LV end-systolic volume), and contractility (P_{sys}/ESV and infarction wall movement velocity) did confirm a positive effect of the additional cell therapy because the changes observed in the standard therapy group failed to reach significance.

Another important factor for interpreting the results is time interval between onset of symptoms and revascularization of the infarct-related artery by angioplasty; this represents a crucial determinant of LV recovery. For patients with acute MI, it has been shown that if the time interval is >4 hours, no significant changes in ejection fraction, regional wall motion, or ESV are observed after 6-month follow-up by echocardiography and angiography.[41] None of our 20 patients was treated by angioplasty within 4 hours after onset of symptoms. Our average time interval was 12±10 hours. Thus, PTCA-induced improvement of LV function can be nearly excluded; indeed, the only mild and nonsignificant changes within the standard therapy group are consistent with the above-mentioned data.[41] In contrast, the cell therapy group showed considerable and significant improvement in the same parameters, which may be attributed to BMC-mediated coronary angioneogenesis and cardiomyoneogenesis.

These results show that transplantation of autologous BMCs, as well as the intracoronary approach, represent a novel and effective therapeutic procedure for the repair of infarcted myocardium. For this method of therapy, no ethical problems exist, and no side effects were observed at any point of time. The therapeutic benefit for the patient's heart seems to prevail. However, further experimental studies, controlled prospective clinical trials, and variations of cell preparations are required to define the role of this new approach for the therapy of acute MI in humans.

References

1. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. *Circulation.* 1990;81:1161–1172.
2. Ertl G, Gaudron P, Hu K. Ventricular remodeling after myocardial infarction: experimental and clinical studies. *Basic Res Cardiol.* 1993;88:125–137.
3. Quaini F, Urbanek K, Beltrami AP, et al. Chimerism of the transplanted heart. *N Engl J Med.* 2002;346:5–15.
4. Leor J, Patterson M, Quinones MJ, et al. Transplantation of fetal myocardial tissue into infarcted myocardium of rat: a potential method for repair of infarcted myocardium? *Circulation.* 1996;94(suppl II):332–336.
5. Murry CE, Wiseman RW, Schwartz SM, et al. Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest.* 1996;98:2512–2523.
6. Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med.* 1998;4:929–933.
7. Tomita S, Li RK, Weisel RD, et al. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation.* 1999;100(suppl II):247–256.
8. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? *Cell.* 2001;105:829–841.
9. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell.* 2001;105:369–377.
10. Goodell MA, Jackson KA, Majka SM, et al. Stem cell plasticity in muscle and bone marrow. *Ann N Y Acad Sci.* 2001;938:208–218.
11. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* 2001;410:701–705.
12. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A.* 2001;98:10344–10349.
13. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med.* 2001;7:430–436.
14. Tomita S, Mickle DA, Weisel RD, et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J Thorac Cardiovasc Surg.* 2002;123:1132–1135.
15. Menasche P, Hagege AA, Scorsin M, et al. Myoblast transplantation for heart failure. *Lancet.* 2001;357:279–280.
16. Hamano K, Nishida M, Hirata K, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ J.* 2001;65:845–847.
17. Strauer BE, Brehm M, Zeus T, et al. Myocardial regeneration after intracoronary transplantation of human autologous stem cells following acute myocardial infarction. *Dtsch med Wschr.* 2001;126:932–938.
18. Wang JS, Shum-Tim D, Chedrawy E, et al. The coronary delivery of marrow stromal cells for myocardial regeneration: pathophysiologic and therapeutic implications. *J Thorac Cardiovasc Surg.* 2001;122:699–705.
19. Sheehan FH, Bolson EL, Dodge HT, et al. Advantages and applications of the Centerline method for characterizing regional ventricular function. *Circulation.* 1986;74:293–305.
20. Sussman M. Cardiovascular biology: hearts and bones. *Nature.* 2001;410:640–641.
21. Toma C, Pittenger MF, Cahill KS, et al. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation.* 2002;105:93–98.
22. Kamihata H, Matsubara H, Nishiue T, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation.* 2001;104:1046–1052.
23. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science.* 1998;279:1528–1530.
24. Kawamoto A, Gwon HC, Iwaguro H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation.* 2001;103:634–637.
25. Robinson SW, Cho PW, Levitsky HI, et al. Arterial delivery of genetically labelled skeletal myoblasts to the murine heart: long-term survival and phenotypic modification of implanted myoblasts. *Cell Transplant.* 1996;5:77–91.
26. Bittner RE, Schofer C, Weipoltshammer K, et al. Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. *Anat Embryol (Berl).* 1999;199:391–396.
27. Jiang Y, Jahagirdar B, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature.* 2002;20:1–12.
28. Reyes M, Lund T, Lenvik T, et al. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood.* 2001;98:2615–2625.
29. Condorelli G, Borello U, De Angelis L, et al. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. *Proc Natl Acad Sci U S A.* 2001;98:10733–10738.
30. Szilvassy SJ, Bass MJ, Van Zant G, et al. Organ-selective homing defines engraftment kinetics of murine hematopoietic stem cells and is compromised by ex vivo expansion. *Blood.* 1999;93:1557–1566.
31. Gregg DE, Fisher LC. Blood supply to the heart. In: *Handbook of Physiology.* Vol 2. Washington, DC: American Physiological Society; 1963:chap 44, 1517–1584.
32. Strauer BE. Myocardial oxygen consumption in chronic heart disease: role of wall stress, hypertrophy and coronary reserve. *Am J Cardiol.* 1979;4:730–740.
33. Toma C, Pittenger MF, Byrne BJ, et al. Adult human mesenchymal stem cells differentiate to a striated muscle phenotype following arterial delivery to the murine heart. *Circulation.* 2000;102(suppl II):II-683. Abstract.
34. Hendrikx PJ, Martens CM, Hagenbeek A, et al. Homing of fluorescently labeled murine hematopoietic stem cells. *Exp Hematol.* 1996;24:129–140.
35. Ren G, Michael LH, Entman ML, et al. Morphological characteristics of the microvasculature in healing myocardial infarcts. *J Histochem Cytochem.* 2002;50:71–79.
36. Li RK, Mickle DA, Weisel RD, et al. Optimal time for cardiomyocyte transplantation to maximize myocardial function after left ventricular injury. *Ann Thorac Surg.* 2001;72:1957–1963.
37. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res.* 2002;53:31–47.
38. Allgöwer M. *The Cellular Basis of Wound Repair.* Springfield, Ill: Charles C. Thomas; 1956.
39. Xie Y, Zhou T, Shen W, et al. Soluble cell adhesion molecules in patients with acute coronary syndrome. *Chin Med J.* 2000;113:286–288.
40. Soeki T, Tamura Y, Shinohara H, et al. Serial changes in serum VEGF and HGF in patients with acute myocardial infarction. *Cardiology.* 2000;93:168–174.
41. Sheiban I, Fragasso G, Rosano GMC, et al. Time course and determinants of left ventricular function recovery after primary angioplasty in patients with acute myocardial infarction. *J Am Coll Cardiol.* 2001;38:464–471.

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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Viability and differentiation of autologous skeletal myoblast grafts in ischaemic cardiomyopathy.

Hagege AA, Carrion C, Menasche P, Vilquin JT, Duboc D, Marolleau JP, Desnos M, Bruneval P.

Assistance Publique-Hopitaux de Paris, Department of Cardiology, Hopital Europeen Georges Pompidou and INSERM EMI-16, Necker-Paris V University, Paris, France. hagege@club-internet.fr

Autologous skeletal myoblast transplantation might improve postinfarction ventricular function, but graft viability and differentiation (ie, proof of concept) has not been shown. A 72-year-old man had autologous cultured myoblasts from his vastus lateralis injected to an area of transmural inferior myocardial infarction in non-reperfused scar tissue. He showed improvement in symptoms and left-ventricular ejection fraction. When he died 17.5 months after the procedure, the grafted post-infarction scar showed well developed skeletal myotubes with a preserved contractile apparatus. 65% of myotubes expressed the slow myosin isoform and 33% coexpressed the slow and fast isoforms (vs 44% and 0.6%, respectively, in skeletal muscle). Myoblast grafts can survive and show a switch to slow-twitch fibres, which might allow sustained improvement in cardiac function.

PMID: 12583951 [PubMed - indexed for MEDLINE]

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Publication Types:
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Medscape Medical News

Autologous Cell Transplant Helpful in Ischemic **Heart** or Legs

Laurie Barclay, MD

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Nov. 18, 2002 — Autologous cell transplantion may benefit ischemic hearts and legs, according to three presentations on Nov. 18 at the American **Heart** Association's 75th Scientific Sessions held in Chicago, Illinois. Two studies focused on injecting autologous bone marrow cells or autologous skeletal myoblasts into the scarred area of an infarcted **heart**. In another study, injecting autologous bone marrow into ischemic limbs led to new vessel growth, reducing the need for amputation.

"Bone marrow not only can differentiate into **heart** cells, but also smooth muscle cells, connective **tissue** cells and other types of cells to reconstitute the entire structure of a **tissue**," presenter Manuel Galinanes, MD, from the University of Leicester in the U.K., says in a news release. "The benefit [of transplanting bone marrow into scar **tissue** of the **heart**] could be seen only six weeks **after** injection."

In 14 patients with low ejection fraction post-myocardial infarction (MI), autologous bone marrow from the sternum was injected into scarred myocardium during nonemergency coronary artery bypass surgery. **Heart** wall motion measured with echocardiography improved within weeks of treatment, and improvements persisted for at least 10 months **after** treatment.

The regional wall motion score decreased significantly, reflecting less movement abnormality, from a mean score of 2.41 at baseline to 2.16 six weeks **after** treatment and 2.09 ten months **after** treatment. The global wall motion score also decreased significantly from 1.96 before surgery to 1.64 at six weeks, and stabilized at 1.65 **after** 10 months.

Although it is still unproven that bone marrow creates a new cellular infrastructure in **heart** scar **tissue**, "that is the only possible explanation," Galinanes says. "The ability to confirm the presence of scar **tissue** with dobutamine stress echo before surgery, and then confirm it again during surgery, told us that the affected area was dysfunctional and the abnormality was irreversible. We wanted to make sure that we were injecting the marrow into **dead tissue** to help ensure that the injection would not pose any serious risk to the patient."

If additional studies confirm safety and efficacy, Galinanes says that this treatment would be a welcome addition to the post-MI arsenal, which also includes gene therapy, growth factor therapy, and laser treatments.

In a multicenter trial supervised by the U.S. Food and Drug Administration, investigators safely transplanted 16 patients with autologous skeletal myoblasts injected into hearts severely damaged by MI or **heart** failure. Baseline left-ventricular ejection fraction was less than 30%. Eleven patients were undergoing coronary artery bypass surgery and five were having implantation of a left ventricular assist device. Myoblasts extracted from thigh muscle were grown in large quantities in vitro using a controlled cell expansion manufacturing process, and were injected in doses ranging from 10 million to 300 million cells.

"We have been able to **regenerate dead heart** muscle, or scar **tissue**, in the area of **heart** attack without increasing risk of death. Our findings will allow us to move forward with testing if the procedure can improve the contractility of the **heart**," says lead author Nabil Dib, MD, from the Arizona **Heart** Institute in Phoenix. "We found that the transplanted myoblasts survived and thrived in patients. Areas damaged by **heart** attack and cardiovascular disease showed evidence of repair and viability."

Twelve weeks **after** transplant, mean ejection fraction rates improved from 22.7% to 35.8%, or a 58% increase. Echocardiogram, magnetic resonance imaging, and positron emission tomography showed evidence of regeneration in the area of the graft. There were no significant adverse events related to the cell transplant procedure at nine-month follow-up.

The third study showed that bone marrow cells implanted into ischemic legs in patients with peripheral arterial disease (PAD) formed new blood vessels, increased blood flow, and prevented amputation.

"This is the first multicenter and double-blind clinical study to prove the clinical efficacy of growing new blood vessels (angiogenesis) using bone marrow cell transplantation," says lead author Hiroya Masaki, MD, PhD, from Kansai Medical University in Osaka, Japan.

In this randomized trial, 45 patients with PAD received injections of autologous bone marrow mononuclear cells into the calf muscles. Compared with controls who received saline injections, patients who received bone marrow mononuclear cell transplants had a "striking" increase in new capillary formation and in newly visible collateral vessels.

Of 45 treated patients, 31 had an increase in ankle-brachial pressure index in the treated limbs, and 39 had decreased rest pain with improved treadmill endurance. Ischemic ulcers or gangrene healed in 21 of 28 treated limbs.

CD34-cells, which can develop into endothelial progenitor cells, expressed angiogenic growth factors including basic fibroblast growth factor, vascular endothelial growth factor, and angiopoietin-1. Although more research is needed to determine long-term efficacy and safety, "this new angiogenesis therapy using bone marrow cell transplantation may help many patients suffering with ischemic limbs," Masaki says.

AHA 75th Scientific Sessions: Abstracts 111623, 101758, 109801. Presented Nov. 18, 2002.

Reviewed by Gary D. Vogin, MD

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ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation.

Pagani FD, DerSimonian H, Zawadzka A, Wetzel K, Edge AS, Jacoby DB, Dinsmore JH, Wright S, Aretz TH, Eisen HJ, Aaronson KD.

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OBJECTIVES: We report histological analysis of hearts from patients with end-stage heart disease who were transplanted with autologous skeletal myoblasts concurrent with left ventricular assist device (LVAD) implantation. BACKGROUND: Autologous skeletal myoblast transplantation is under investigation as a means to repair infarcted myocardium. To date, there is only indirect evidence to suggest survival of skeletal muscle in humans. METHODS: Five patients (all male; median age 60 years) with ischemic cardiomyopathy, refractory heart failure, and listed for heart transplantation underwent muscle biopsy from the quadriceps muscle. The muscle specimen was shipped to a cell isolation facility where myoblasts were isolated and grown. Patients received a transplant of 300 million cells concomitant with LVAD implantation. Four patients underwent LVAD explant after 68, 91, 141, and 191 days of LVAD support (three transplant, one LVAD death), respectively. One patient remains alive on LVAD support awaiting heart transplantation. RESULTS: Skeletal muscle cell survival and differentiation into mature myofibers were directly demonstrated in scarred myocardium from three of the four explanted hearts using an antibody against skeletal muscle-specific myosin heavy chain. An increase in small vessel formation was observed in one of three patients at the site of surviving myotubes, but not in adjacent tissue devoid of engrafted cells. CONCLUSIONS: These findings represent demonstration of autologous myoblast cell survival in human heart. The implanted skeletal myoblasts formed viable grafts in heavily scarred human